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CYTAMOEBA BACTERIFERA IN THE RED BLOOD CELLS OF THE FROG

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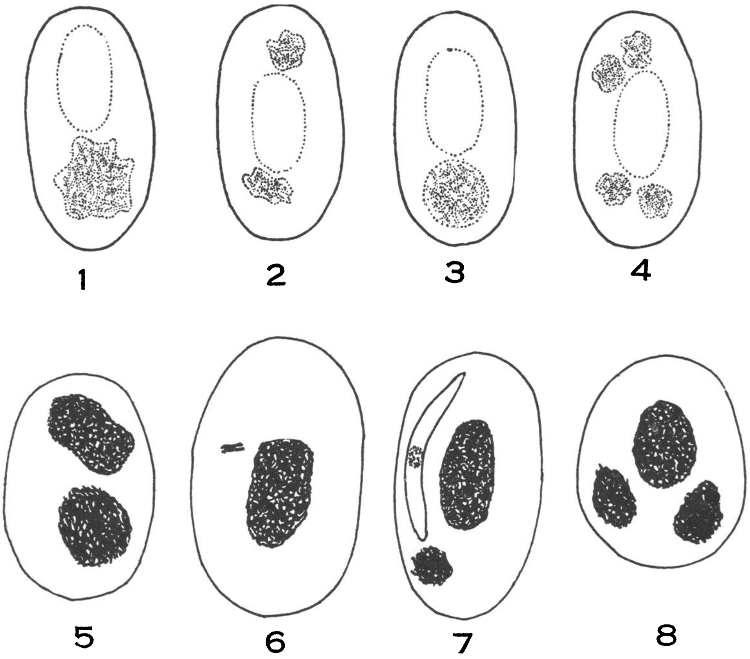
This note is intended to call to the attention of parasitologists and bacteriologists the presence in the red blood cells of frogs in this country of a most interesting organism to which the name *Cytamoeba bacterifera* was given by Labbé in 1894.

Cytamoeba bacterifera was found in the red cells in the peripheral blood of two specimens of *Rana clamitans* and five specimens of *R. catesbiana* collected at Cold Spring Harbor, Long Island, N. Y., in the summer of 1919. It was absent from the other frogs and toads examined; namely, *Rana palustris, R. sylvatica, R. pipiens, Hyla versicolor, Bufo americanus* and *Scaphiopus holbrookii.* In every case the host was also parasitized with trypanosomes, hemogregarines, and several other forms that represent either undescribed species or stages in the life cycles of parasites known only as adults. Freshly drawn blood was studied as well as material fixed and stained by Wright's method and by the Schaudinn-iron-haematoxylin method. The frogs were kept alive in the laboratory for a considerable period and their blood examined at intervals of a few days; several of them were kept in this way for three months.

The importance of studying specimens of Cytamoeba while alive and in freshly drawn blood was soon demonstrated, since movement ceases shortly after being taken from the frog's body and the appearance in stained preparations is far from normal. A typical, fully grown specimen as seen when alive and active is shown in figure 1. It measures about 7μ in diameter and occupies a position at one end of the red cell, sometimes pushing the nucleus out of its usual central position. The entire parasite is active as well as the bacilliform bodies within it. It resembles very much a trophozoite of the tertian malarial parasite, *Plasmodium vivax,* throwing out and withdrawing pseudopodia on all sides. At the same time the bacilliform bodies are moving about rapidly within, creating a flame-like appearance. After a few minutes the movements of the parasite cease and a stationary, rounded shape is assumed (Fig. 3), but the bacilliform bodies continue to move about within, often for several hours. Finally all movement of both parasite and bacilliform bodies ceases. Many stages in the growth of the parasite are present within the red blood cells of a frog at the same time. The first recognizable stages appear like minute vacuoles frequently situated at the side of the nucleus of the red cell instead of at one end as later. Only one or a few bacilliform bodies may be present at this time (Fig. 6). As growth proceeds the size of the Cytamoeba increases as well as the number of bacilliform bodies. Fully grown parasites differ with respect to the number of these bodies, some being supplied



All figures were drawn with a camera lucida × 1440.

Fig. 1. Large, living, active specimen of *Cytamoeba bacterifera* within red blood cell of bullfrog. The nucleus of the blood cell is pushed out of its normal position. The parasite has thrown out pseudopodia and is filled with active bacilliform bodies.

Fig. 2. Red blood cell containing two living, active specimens.

Fig. 3. Red blood cell containing one living specimen that has rounded up and has ceased to throw out pseudopodia. The bacilliform bodies within are still active.

Fig. 4. Red blood cell containing four living, active specimens.

Fig. 5. Red blood cell containing a large specimen. This and figures 6, 7 and 8 were drawn from material fixed and stained by Wright's method.

Fig. 6. Red blood cell containing a very young specimen with only two bacilliform bodies.

Fig. 7. Red blood cell infected with both Cytamoeba and Haemogregarina.

Fig. 8. Red blood cell containing two specimens of Cytamoeba.

with more than others. Blood specimens from frogs that were kept in the laboratory were examined at intervals for three months but no other stages in the life history of the Cytamoeba were found and no great differences were observed in the percentage of infection of red blood cells or in the sizes of the organisms present. For example, in a bull frog (No. 23) the percentage of infection of red blood cells ranged from 4 to 7 at different times during a period of three months. The infection in other specimens ranged from 1% up to 12%. In a few cases red blood cells contained two Cytamoebas (Figs. 2 and 8) and in one case four were present (Fig. 4). Several cells were infected with both Cytamoeba and Haemogregarina (Fig. 7). No visible difference was observed between the size and contents of the infected and noninfected red cells either in fresh blood or in the fixed and stained material such as is sometimes so conspicuous when cells are parasitized by large specimens of Haemogregarina or Karyolysus. No specimens of Cytamoeba were found free in the blood as reported by Labbé (1894) and Laveran (1899).

Specimens fixed and stained by Wright's method present an entirely erroneous appearance. The amoeboid or rounded shape of the parasite is entirely lost due probably to drying and an irregular mass of bacilliform bodies is left. These stain deeply and are quite conspicuous. The appearance of the parasite and its contained bodies is indicated in the accompanying figures (Figs. 5-8). Specimens fixed in Schaudinn's solution and stained with iron-haematoxylin are unsatisfactory. They keep their rounded shape, but do not stain well.

Several interesting questions regarding Cytamoeba remain to be solved. Is Cytamoeba an organism or only a vacuole in which bacteria live? If it is an organism is it an "adult" stage or a developmental stage of an unknown species or of a known species such as *Haemogregarina ranarum* or *Trypanosoma rotatorium*? If it is a stage of an unknown species, what stage in the life cycle does it represent and in what part of the body do the other stages occur? Are the bacilliform bodies bacteria? If so, how does the Cytamoeba become infected by them?

Kruse (1890), probably the first to figure this parasite, speaks of it not as an organism but as a space in the blood. Gabritchewsky's (1890) attention was called to it by Metchnikoff who found it in the blood of frogs infected with *Haemogregarina ranarum.* His efforts were chiefly directed toward an investigation of the "bacteria," nevertheless the "petite boule protoplasmique transparente" which contained these bacteria was considered "un être complètement, étrange à l'hématie, une espèce d'amibe, ou de larve amibiforme envahie ellemême par un microbe bactérien." Four uninfected frogs were inoculated with the spleen, liver, kidney and bone marrow of a frog containing both Haemogregarina and Cytamoeba. Three of the frogs were later found to possess both types of parasites; in the fourth animal only the Cytamoebae were present. Autopsies revealed the Cytamoebae in the spleen, and bone marrow. A small number of trypanosomes were noted in all frogs containing the other parasites. Culture experiments gave negative results. Gabritchewsky suggests that perhaps "le corpuscle amibiforme" is a stage in the development of Haemogregarina.

The name *Cytamoeba bacterifera* we owe to Labbé (1891, 1894). This investigator recognized it as a new type of organism and placed it in the genus Cytamoeba, a generic name proposed previously by Danilewsky (1890) for the malarial parasite. A parasite of the red blood cells of *Hyla viridis* described by Grassi (1882) and considered by him analogous to the malarial organism was placed by Labbé provisionally in the same genus and given the name *Cytamoeba grassi.* Labbé describes what he thought were stages of Cytamoeba in fission and spore formation, and specimens that he found free in the blood.

Wasielewski (1896) accepted Labbé's views regarding the protozoan affinities of *Cytamoeba bacterifera* and placed it in the order Acystosporidia, family Haemamoebidae, along with the genera Proteosoma, Haemamoeba, and Dactylosoma. Cytamoeba was next described by Ziemann (1898) who noted the amoeboid movement of vacuole-like bodies within the red blood cells of the frog, and observed the bacilliform bodies contained in them but was not convinced that the body he saw was really an amoeboid organism.

Laveran (1899) also observed *Cytamoeba bacterifera* both in fresh and in stained preparations. He named the bacilliform bodies *Bacillus krusei* and concluded that the bacteria were not contained in a parasitic ameboid organism, because (1) no movement of the vacuole was observed; (2) the bacilliform bodies moved actively as though in a liquid and not in protoplasm; (3) isolated bacilli were encountered, without a surrounding clear area; (4) the vacuoles did not become stained and no nucleus could be found; (5) after the dissolution of the protoplasm of the red blood cell, the bacteria were isolated; and (6) no reproduction of the "ameba" was observed. Laveran did not succeed in cultivating *Bacillus krusei.* Several of Laveran's contentions do not hold since (1) the "vacuole" certainly undergoes amoeboid movement, (2) protoplasm within certain amoebae is liquid as is probably also true of Cytamoeba and the active movement of the bacilliform bodies is possible in this medium, (3) there is no reason why bacilli should not exist outside of the organism, (4) failure to find a nucleus does not prove that one is not present since it may be obscured by the bacilliform bodies, (5) the protoplasm of the organism as well as that of the red cell may have been dissolved, and (6) the reproduction of the "ameba" may occur in the internal organs.

More recently Dutton, Todd and Tobey (1907) have described several organisms that were found by them in the red blood cells of frogs in Africa, some of which may have been Cytamoebas; and Carini (1910) has attempted to classify similar bodies found in the Brazilian frog, *Leptodactylus ocellatus.* These were apparently observed only in stained specimens and hence were unsatisfactory for study. Certain of them are called by Carini "elements bacilloides"; a second type consists of bacilliform bodies similar to Bacillus krusei but thinner and shorter, and a third type resembles Cytamoeba.

After studying infected red blood cells of the frog both living and fixed and stained and reviewing the literature on this subject the tentative conclusion is reached that *Cytamoeba bacterifera* is a stage in the life cycle of a protozoan parasite and that living within it either as hyperparasites or in symbiosis is a bacillus named by Laveran *Bacillus krusei.* The writer hopes at some future time to add more to our knowledge of this interesting organism.

LITERATURE CITED

Carini, A. 1910.—Sur quelques parasites semblables a des bacilles rencontres dans les hématies du Leptodactylus ocellatus. Ann. Inst. Pasteur, 24: 1-5; 2 pl.

Dutton, Todd and Tobey. 1907.—Concerning Certain Parasitic Protozoa Observed in Africa. Ann. Trop. Med and Parasit., 1: 334.

Gabritchewsky. 1890.—Contribution a l'etude de la parasitologie du sang. Ann. Inst. Pasteur, 4: 440-445.

Kruse, W. 1890.—Ueber Blutparasiten. Archiv. path. Anat. Phys., 120: 541-560.

Labbé, A. 1894.—Recherches zoologiques et biologiques sur les parasites endoglobulaires du sang des vertébrés. Théses. Paris, 208 pp., 10 pl.

Laveran, A. 1899.—Sur le bacille parasite des hématies de Rana esculenta. Compt. rend soc. biol., 51: 355-358.

Wasielewski, T. von. 1896.—Sporozoenkunde. 162 pp., 111 figs. Jena.

Ziemann, H. 1898.—Ueber Malaria und andere Blutparasiten. 191 pp., 5 pl. Jena.